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(51) International Patent Classification[6]: C07H 19/06, 19/10, 19/20, 19/16, A61K 31/70	A1	(11) International Publication No.: WO 96/11204 (43) International Publication Date: 18 April 1996 (18.04.96)
(21) International File No.: PCT/DE95/01412 (22) International Application Date: 5 October 1995 (5.10.95) (30) Priority date: P 44 36 995.6 7 October 1994 (7.10.94) DE 195 18 216.2 10 May 1995 (10.05.95) DE		(81) Designated countries: JP, US, European Patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published *With international search report.*

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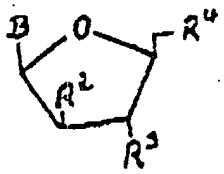
(54) Title: NOVEL β-L-NUCLEOSIDES AND THEIR USE

(57) Abstract: [In English and German]

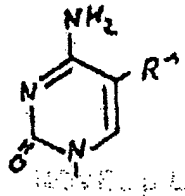
NOVEL β-L-NUCLEOSIDES AND THEIR USE

Description

The invention relates to novel β-L-nucleosides of the general formula



wherein

B =  , guanine, 2-aminopurine;

R^1 = H, methyl, halogen, formyl, hydroxymethyl, ethyl, chloroethyl;

R^2 = H, OH;

R^3 = F, OH; when R^2 = H, then R^3 = F, when R^2 = OH, then R^3 = OH

R^4 = OH, O-acetyl, O-palmitoyl, alkoxycarbonyl, phosphonate,

mono-, di-, triphosphate, and another protective group,

which

In a subsequent reaction may be converted into the hydroxy group,

and their use as active pharmaceutical substances and agents for the prophylaxis and/or treatment of infections caused particularly by the hepatitis B virus (HBV) and the HIV (human immunodeficiency virus). Fields of application of the invention are medicine and the pharmaceutical industry.

The HBV is the triggering agent for hepatitis B, an infectious disease which strikes some 200 million people worldwide and the chronic form of which is associated with an increased risk of primary liver carcinoma, which in China alone results in approximately one million new cases of tumorous disease each year.

No effective and well-tolerated antiviral therapy is yet available. The use of adenine arabinoside monophosphate and acyclovir has been limited to a few clinical studies, due to considerable side effects at this time and the but partial and temporary success of treatment (Alexander et al., British Medical Journal 292, 915 (1986)). Only with interferon has longer-lasting therapeutic success recently been obtained in approximately 50% of cases treated.

The treatment of HIV infections (AIDS), which, as late sequelae of infection of T4 lymphocytes with HIV, results in the breakdown of immunological resistance, must be viewed as similarly unsatisfactory. Previous antiviral therapy with azidothymidine and recently with didesoxyinosine, which is better tolerated, has delayed but cannot prevent the fatal outcome of the immune deficiency syndrome.

A variety of nucleoside analogs, which are disclosed in the following documents, are novel potentially active agents:

1. EP 0,277,151 and EP 0,254,268 – 3'-Fluoro nucleosides of adenine, guanine, cytosine and thymine.
2. WO 89/01776 – 2'-Fluoroarabinofuranosyl-5-ethyluracil.
3. EP 0,302,760 – 2',3'-Didesoxy nucleosides of various purine derivatives.
4. EP 0,322,384 and EP 0,409,227 – Sugar-modified purine and pyrimidine nucleosides.

5. EP 0,330,992 – Cyclopentane derivatives of purines and pyrimidines.

6. EP 0,434,450, EP 0,349,242, US 4,999,428 and WO 91/00282 – Carbocyclic nucleosides of purine derivatives.

7. EP 0,433,898 – Oxetane derivatives of purines and pyrimidines.

8. EP 0,442,757 – 3'-Fluoro nucleosides.

All nucleosides described here are present in D form.

L-nucleosides, the enantiomers of naturally occurring D-nucleosides, have long been deemed not enzymatically metabolizable and hence ineffective in biologic systems. A break with this dogma was made in 1992 by the findings of Spadari et al., who showed that while β-L-thymidine is not converted by cellular TdR kinase, a substrate of the corresponding enzyme of Herpes simplex virus 1 is (Spadari et al., J. Med. Chem. 1992, 35, 4214-4220). In the period following, a variety of β-L-nucleoside analogs, such as for example: β-L-dideoxycytidine (L-ddC) (M. Mansuri et al., Bioorg. Med. Chem. Lett. 1991, 1, 65-68), β-L-5-fluorodideoxycytidine (L-FddC) and β-L-5-fluoro-dideoxy-uridine (L-FddU) (T.-S. Lin et al., J. Med. Chem. 1994, 37, 798-803), β-L-3-thiacytidine (L-3TC) (C. N. Chang et al., J. Biol. Chem. 11992, 267, 22414-22420) and β-L-5-fluorothiacytidine (L-FTC) (P. A. Furman et al., Antimicrob. Agents Chemother. 1992, 36, 2686-2692) were prepared in pure form or purified. These compounds were compared with the corresponding enantiomers with respect to their antiviral activity on HBV and HIV replication and their antiproliferative toxicity.

Additional syntheses of L-nucleosides are described in

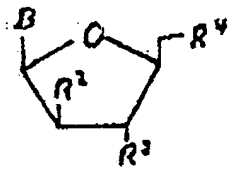
- A. Holy, Collect. Czech. Chem. Commun. 1972, 37, 4072=4087
- M. J. Robins et al., J. Org. Chem. 1970, 35, 636-639

- Y. Abe et al., Chem. Pharm. Bull. 1980, 28, 1324-1326.

However, no compounds that are modified by fluorine at the 3' position of the sugar group and contain an L-arabinofuranosyl group have been disclosed.

The object of the invention is to develop novel antiviral active β-L-nucleosides which are effective against hepatitis B and HIV infections and which, with good tolerance and low toxicity, have a high effectiveness against these infections.

Surprisingly, β-L-nucleosides of the general formula



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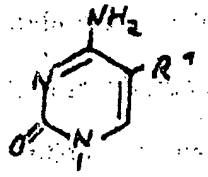
B = 

, guanine, 2-aminopurine;

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R^4 = OH, O-acetyl, O-palmitoyl, alkoxycarbonyl, phosphonate,

mono-, di-, triphosphate, and another protective group,

which

in a subsequent reaction may be converted into the hydroxy group, exhibit high antiviral activity.

3'-Fluoro-modified compounds of formula I, among them β-L-2',3'-didesoxy-3'-fluorocytidine, β-L-2',3'-didesoxy-3'-fluoro-5-methylcytidine, β-L-2',3'-didesoxy-3'-fluoro-5-chlorocytidine and β-L-2',3'-didesoxy-3'-fluoroguanosine are especially active. β-L-5-Methylcytosine arabinoside also exhibits high activity.

The compounds according to the invention are prepared according to a method known per se by condensation of the sugar portion and heterocycle and by alteration of the L-ribosyl group.

Thus, for example, L-ribose is acetylated and condensed with the heterocyclic base. The resulting L-ribonucleoside is deoxygenated and then modified, for example fluorinated, in the 3'-position. The starting material L-ribose may be obtained in simple fashion by epimerization of L-arabinose, owing to which preparation of the compounds according to the invention is also economically affordable.

The invention will be explained in detail below by examples.

Examples:

1. Synthesis of β-L-2',3'-didesoxy-3'-fluoro-5-methylcytidine

A solution of 1-(5-O-acetyl-2,3-didesoxy-3-fluoro-β-L-ribofuranosyl)thymine (788 mg, 2.8 mmol), 1,2,4-triazole (400 mg, 5.6 mmol) and 4-chlorophenyldichloro-phosphate (0.67 ml, 4.2 mmol) in pyridine (25 ml) remains at room temperature for five days. Then concentrated ammonia solution (40 ml) is added to the dark brown reaction mixture [(W.L.J. Sung, J. Chem. Soc. Chem. Commun. 1089 (1981)]. After 10 hours the solvent

is removed in vacuo. The remaining residue is dissolved in 50 ml water and purified by column chromatography on Dowex WX 8 (H' form, 50 ml) with water (1000 ml) and 5% ammonia solution (300 ml) as eluent. The preliminary compound is obtained from the ammoniac eluate as crude product. Separation by column chromatography of the crude material on silica gel 60 (0.063-0.2 mm) (Merck) with chloroform (15% methanol) supplies β-L-2',3'-didesoxy-3'-fluoro-5-methylcytidine, which is obtained in methanol with little HCl as hydrochloride (314 mg, 41% yield).

MS: m/2 243 (M^+ –HCl);

UV (H_2O, pH = 7): λ_{max} 278 nm (7430).

2. Determination of antiviral activity of β-L-2',3'-didesoxy-3'-fluoro-5-methyl-cytidine (L-FMetCdR)

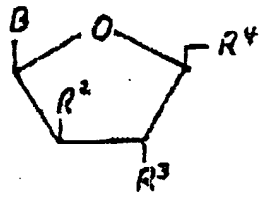
Human hepatoblastoma cells, which had been transfixed with the hepatitis B virus (HBV) (HepG2 2.2.15 cells) and produce the virus permanently [(Sells et al., Proc. Natl. Acad. Sci. USA 84, 1005 (1987)] were incubated in RPMI 1640 medium, to which 2 mM glutamine and 10% fetal calf serum were added. After 5 days' incubation the medium was renewed and L-FMetCdR added to the batch in various concentrations. The medium was replaced every two days and at the same time the inhibiting agent was also replaced.

After 8 days' incubation of the cells with L-FMetCdR the medium was centrifuged and the viruses precipitated from the supernatant with 10% polyethylene glycol, the HBV-DNA therein purified and quantified by means of dot-blot analysis [(E. Matthes et

al. Antimicrob. Agents Chemother. 34, 1986 (1990)]. L-FMetCdR is capable of completely suppressing synthesis of the HBV. The concentration of inhibiting agent, that reduces the quantity of HBV-DNA released by the cells into the medium by 50% is less than 0.2 μM. 50% inhibition of proliferation of HepG2 2.2.15 cells (CD_{50}) is obtained only at concentrations greater than 400 μM.

Claims

1. Novel β-L nucleosides of the general formula



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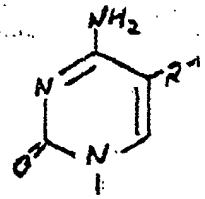
B =  , guanine, 2-aminopurine;

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mono-, di-. triphosphate, and another protective group,

which

in a subsequent reaction may be converted into the hydroxy group.

2. β-L-2',3'-Didesoxy-3'-fluorocytidine

3. β-L-2',3'-Didesoxy-3'-fluoro-5-methylcytidine

4. β-L-2',3'-Didesoxy-3'-fluoro-5-chlorocytidine

5. β-L-2',3'-Didesoxy-3'-fluoroguanosine

6. β-L-5-Methylcytosine arabinoside

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AFFIDAVIT OF ACCURACY

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) ss.:
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I, the undersigned, being duly sworn, depose and state:

I am qualified to translate from the German language into the English language by virtue of being thoroughly conversant with these languages and, furthermore, having translated professionally from German to English for more than 10 years;

I have carefully made the translation appearing on the attached and read it after it was completed; and said translation is an accurate, true and complete rendition into English from the original German -language text, and nothing has been added thereto or omitted therefrom, to the best of my knowledge and belief.

TRANSLATION ACES, INC.
BERTRAND LANGUAGES INC.

Subscribed and sworn to before me

this 19 day of March , 2002

KARYN L. TAGENS
Notary Public, State of New York
No. 31-4680695
Qualified in New York County
Commission Expires Oct. 31 2002